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Review

Polyamines in food–implications for growth and health

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Different types of food (fruits, vegetables, meat, and milk products) were analyzed by high pressure liquid chromatography to determine their polyamine (putrescine, spermidine, and spermine) contents. All foods contained some polyamines, although the concentrations in different individual food components were variable. As was established earlier using ^{14}C-labeled putrescine, spermidine, and spermine, polyamines are readily taken up by the gut and enter the systemic circulation. Food appears to constitute a major source of polyamines for humans and animals. The distribution of polyamines in the body, as determined by measuring the accumulation of ^{14}C-spermidine in different tissues of the rat, was correlated with the metabolic activity and growth of particular organs. Thus, phytohemagglutinin induced both extensive hyperplastic growth and the preferential accumulation of labeled spermidine in the gut. Correspondingly, when skeletal muscle growth was promoted by the β-antagonist, clenbuterol, ^{14}C-spermidine was sequestered by the hind leg gastrocnemius muscle. It is concluded that food polyamines are not only necessary for normal body metabolism, but are also used and directed preferentially to tissues and organs that have been stimulated to grow by metabolic signals.

Keywords: polyamines; putrescine; spermidine; spermine; diet; food

Introduction

Polyamines (putrescine, spermidine, and spermine) are indispensable components of all living cells. Although they have long been known to be essential for growth, their exact biological role in cell metabolism is still unclear. Polyamines are flexible polycations, fully protonated at physiological pH. In contrast to metal ions, which have localized charges, polyamines can form bridges between distant negative charges because their positive charges are distributed along a hydrocarbon chain. As a result, polyamines can fulfill uniquely specific functions in cells.[1,2]

Due to the diversity of the roles of polyamines in cellular metabolism and growth, they are required in rapidly growing tissues in large amounts.[3,4] Indeed, the importance of polyamines in tumor growth is widely recognized, and inhibiting polyamine biosynthesis in cancerous tissues is a major target for scientists involved in polyamine research.

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Because all cells have the capacity to synthesize polyamines,[2,3] it has been suggested that they are produced in situ according to need. However, in some cases the synthetic capacity of cells or organs is not sufficient to satisfy all requirements. For example, although the phytohemagglutinin (PHA)-induced gut growth in rats[5–7] was coincident with the accumulation of substantial amounts of polyamines in the small intestine,[5] this was partly the result of increased biosynthesis[6] via ornithine decarboxylase (ODC, EC 1.1.4.17), the first enzyme of polyamine biosynthesis; and partly of the stimulation of the basolateral uptake of polyamines, particularly spermidine, from the circulation.[7] Thus, in this instance ODC was not responsible for most of the polyamine accumulation. This was confirmed in studies using a substrate analogue, α-difluoromethylornithine (DFMO),[8] an irreversible inhibitor of ODC. Although this drug is an efficient blocker of the proliferation of cells in culture,[9] in vivo it does not always completely stop the growth or regeneration of tissues or block the growth of tumors in mammals.[9,10] In line with this, DFMO also failed to completely block the PHA-induced small intestinal hyperplasia, confirming that ODC induction was not always a prerequisite for growth or polyamine accumulation.[8] Accordingly, polyamines may also be derived from

external sources, of which the diet appears to be the most likely.

Polyamines are essential for the maintenance of the high metabolic activity of the normally functioning and healthy gut. Unfortunately, their presence in food or, even more importantly, their physiological effects on the gut, including their involvement in the repair of gut damage caused by the deleterious components of food and/or bacteria, have in the past been totally neglected. In spite of the undoubted importance of polyamines in the growth processes, it is difficult to find any information in nutritional handbooks on the polyamine content and composition of even the most common food regularly consumed by humans.

The aims of this paper are to provide quantitative data on the concentration of individual polyamines in major and everyday foods and to understand how they are taken up by the gut and distributed and stored in different organs of the body to support growth.

Materials and methods

Analysis of food polyamine content

Three separate samples of the different foods were collected from the institute's kitchen at different times for analysis. Four aliquots of each (1.0–1.5 g or mL), with 1,7-diaminoheptane added as an internal standard, were homogenized in 5–7 mL of 10% wt/vol aqueous perchloric acid (PCA) in a Janke-Kunkel homogenizer at 20–23,000 rpm for 1 min, left to stand on ice for 15 min, and centrifuged at 1,000*g* for 10 min. The residue was rehomogenized with the same volume of distilled water and treated as before. The supernatants were combined and diluted (1:1.5) with distilled water. These samples were analysed by high performance liquid chromatography (HPLC) according to the method of Seiler and Knödgen[11] and corrected for the internal standard. The sensitivity of the assay, taking into consideration the dilution factors and recovery, was between 0.1 and 0.6 nmol/g or mL depending on the type of the sample.

Animals

One-month-old male Hooded-Lister rats weighing about 80 g were pre-fed on powdered lactalbumin (Sigma Chemical Co., St. Louis MO USA; 10% protein) control diet ad libitum for 3 days. This control diet was fully supplemented with minerals and vitamins, and was essentially the same as described,[12] except that instead of egg albumin or casein lactalbumin was used. This diet contained a daily dose of 66 ± 19 nmol putrescine and 60 ± 12 nmol spermidine.[5] The animals were divided into three groups, each containing four rats. Two of these groups were fed the lactalbumin diet (5.5 g/rat/day) for 4 more days with or without the inclusion of 22 μg clenbuterol/rat/day, (4 mg/kg^{-1} diet; Lederle Laboratories, Gosport, Hants, UK), a β-adrenoreceptor agonist that induces skeletal muscle hypertrophy.[13,14] To stimulate small intestinal growth, another group was fed a kidney bean diet (5% kidney bean protein + 5% lactalbumin)[15] for the same length of time. In this diet the active growth factor is the lectin component of the kidney bean.[16] The food consumption of rats in all groups was restricted to 5.5 g/rat/day, which was the natural voluntary intake of the bean group. This amount of food contained 30 ± 2 nmol putrescine, 174 ± 29 nmol spermidine, and 188 ± 22 nmol spermine.[5] The animals had access to acidified water ad libitum. At the end of the experiment, rats, fasted overnight, were fed 2 g of the appropriate diet 2 hr before killing them by decapitation. Distribution of polyamines in the different organs of the body was measured by injecting the rats intraperitoneally with 9.9 nmol (3.3×10^6 dpm) ^{14}C-spermidine (Amersham International Plc, Amersham, Buckinghamshire, UK) precisely 1 hr before killing them under ether anesthesia. Rats were dissected; their tissues collected, weighed, and prepared for the measurement of polyamine uptake.

Polyamine uptake studies

After removal, the small intestine of the rats was washed with ice-cold saline, six sections of 2 cm were cut out from each at 3, 7, 21, 41, 63, and 81 cm from the pyloric end, weighed, and placed into NE 265 scintillation fluid (NE Technology Limited, Edinburgh, UK). After 2 days of extraction, the sections were counted for radioactivity as previously described.[7] A part of the colon, cecum, pancreas, lungs, spleen, and the thymus were also extracted for 2 days with NE 265. The uptake of polyamines by the individual organs was calculated pro rata from the amounts of radioactivity incorporated into the sections during the 1-hr labeling period. Some of the tissues had to be digested before measuring their radioactivity. Accordingly, the right gastrocnemius muscle, one of the kidneys, the stomach, a 100–150 mg piece of the liver, and about 500 mg of the dried ground carcass of the rats were digested with 1 mL of NCS tissue digester (Amersham International Plc, Buckinghamshire, UK) at 60° C for 16 hr, after which 10 mL of scintillation liquid and 30 μL of concentrated acetic acid were added to each sample and counted for radioactivity. The label incorporated into the tissues was calculated from the weight ratio of the piece to the total. The individual variation in the distribution of label within a group was 5–7%.

Statistics

Food polyamine data are expressed as ranges obtained from the analyses of three parallel samples.

Results

Polyamine analysis of food samples

Food samples collected from the institute's kitchen with or without cooking or other methods of preparation were homogenized in PCA and their polyamine content determined by HPLC. All food samples analyzed contained some polyamines but in different quantities *(Table 1)*. Fish (except trout) had low spermidine and spermine but high putrescine contents compared with chicken, pork, and beef. In these, the most prominent polyamine was spermine. Milk products contained mainly putrescine and spermidine. The polyamine content of cow's milk was surprisingly low. Vegetables contained more spermidine and putrescine than spermine. The putrescine content of fruits was high in relation to the amounts of spermidine and spermine.

Distribution of ^{14}C-spermidine in the body

As shown earlier,[7] when rats were intubated intragastrically with ^{14}C-labeled putrescine, spermidine, or spermine, the polyamines were readily taken up by

Table 1 Polyamine content (nmol/g wet weight or nmol/mL) of some food consumed by humans

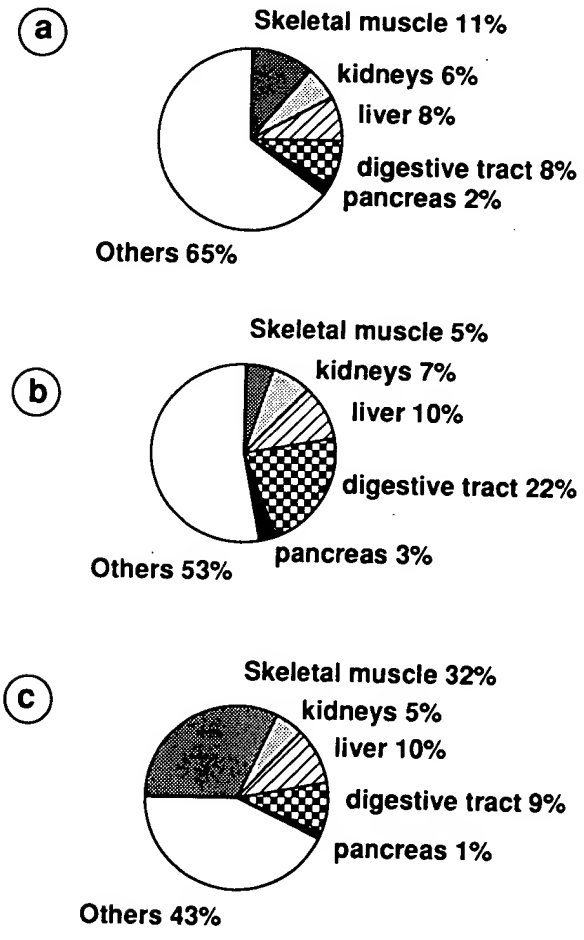
Food type:	Polyamines (nmol/g or mL)		
	Putrescine	Spermidine	Spermine
Meat:			
cod	300–337	7–11	15–32
trout	21–22	24–35	22–30
fish sausage	180–185	27–29	43–45
chicken	32–33	63–65	291–296
pork, lean	34–35	20–34	149–348
pork bacon	45–46	27–41	100–249
pork ham, smoked	45–49	14–61	199–249
pork ham, roasted	101–103	41–43	199–299
meat sausage	157–165	40–44	119–128
Beef, lean, raw	63–67	126–136	152–208
beef, cooked	22–32	39–47	113–165
ground beef	100–101	487–503	229–235
Milk products:			
cheddar, matured	7409–7427	1361–1392	115–198
cheddar, fresh	115–227	557–751	118–194
full cream milk	1	1–3	1–3
semi-skimmed milk	1–2	2–4	1–2
Cereals:			
whole bread	6–10	147–189	35–45
white bread	17–21	57–59	17–19
bran, rivita	7–11	28–64	2–4
pasta, cooked	11–12	48–50	52–64
rice, cooked	11–15	9–11	40–50
Vegetables:			
cabbage leaves	4–18	22–35	16–18
onion	62–82	38–56	4–6
potato	108–112	76–78	14–16
potato, cooked	229–261	101–109	24–28
mushroom	1–2	236–279	4–6
carrot	14–20	53–57	10–14
cauliflower	35–51	150–192	48–64
lettuce	37–55	29–57	0
tomato	106–1386	11–17	0
cucumber	36–37	10–11	1–3
radish	2–3	3–4	6–8
soya bean	18–74	229–428	147–170
red kidney bean	4–5	131–138	113–127
green beans, cooked	49–61	53–61	23–27
green peas, cooked	61–67	428–470	166–355
Fruits:			
orange	1081–1579	61–67	0
orange, canned	307–341	5–7	0
apple	5–19	15–19	0
pears	268–275	208–524	40–244
grapefruit juice	1120	0	0

Data are expressed as range of polyamines measured in quadruplicate on three different samples of the same food from the institute's kitchen. Food samples were raw unless indicated otherwise. The limit of detection was between 0.1 and 0.6 nmol/g or mL. For the method of sample preparation and analysis see Materials and Methods section.

the gut from the lumen, passed into the systemic circulation very quickly, and accumulated in tissues. To investigate the distribution of polyamines in the body, rats were injected intraperitoneally with ^{14}C-labeled spermidine and the accumulation of the label in individual organs was determined. Radioactivity was detected in every tissue examined, indicating that spermidine was distributed to all organs of the body. However, the pattern of distribution differed according to the diet fed to the rats.

In rats fed the lactalbumin (control) diet and injected with 9.9 nmol ^{14}C-spermidine, the distribution of label after 1 hr (as percent of the dose) was as follows: stomach tissue, 1 ± 0.1%; stomach contents, 0.1%; small intestine, 5.5 ± 0.7%; small intestinal lumen, 0.2 ± 0%; cecum, 0.8 ± 0.1%; colon, 0.6 ± 0.1%; left hind leg gastrocnemius muscle, 0.1 ± 0.01%; kidneys, 5.8 ± 0.6%; and liver, 1.7 ± 0.2%. Thus, taking the gastrocnemius as a representative of skeletal muscle, 11.3 ± 1.5% of the label accumulated in a tissue, which accounts for about 40% of the total body weight. The distribution of the ^{14}C-spermidine in different organs is presented in *Figure 1a*.

In rats fed a diet containing kidney bean,[7,16] which induces hyperplastic growth of the bowel, more radioactivity was retained by the gastrointestinal tract than in the control animals. Thus, tissue radioactivity expressed on the basis of the injected dose of ^{14}C-spermidine was: stomach, 1.3 ± 0.1%; stomach contents, 0.16 ± 0%; small intestine, 15.5 ± 1.1%[7]; its luminal contents, 0.5 ± 0%; cecum, 2.1 ± 0.2%; colon, 2.0



Figur 1 Distribution of ^{14}C-spermidine in different organs of rats fed on (a) lactalbumin-, (b) kidney bean-, or (c) clenbuterol-containing diets for four days. Spermidine was given i.p. 1 hr before killing.

± 0.1%; pancreas, 2.6 ± 0.3%[17]; and left gastrocnemius muscle, 0.06 ± 0.01.

On kidney bean diet, the dry weight/100 g dry body weight of the small intestine was 4.10 ± 0.11. This value for the lactalbumin-fed control was 2.53 ± 0.13. The rate of spermidine uptake in the small intestine of bean-fed rats was disproportionally higher than the increase in the relative weight of the tissue under the same conditions. The dry weight, but not the dry weight/100 g body weight, of the hind leg gastrocnemius muscle decreased in bean-fed rats, which was accompanied by a 40% loss in spermidine uptake in this tissue *(Figure 1b)*.

When growth of the skeletal muscle was induced by the β-agonist, clenbuterol, the hind leg gastrocnemius muscle of these rats contained significantly more radioactivity (0.3 ± 0.02%) than that of the controls or the kidney bean-fed group. The total label in the skeletal muscle calculated from the ratio of hind leg gastrocnemius/total skeletal muscle of the rats was increased to 32 ± 2%. However, the gain in the dry weight/100 g body weight of the hind leg gastrocnemius muscle (the difference between the lactalbumin-fed control value of 0.39 ± 0.01 and 0.46 ± 0.02 in clenbuterol-fed rats) was less extensive. In contrast, the gastrointestinal tract of the clenbuterol-treated animals contained less radioactivity than that of the kidney bean group; counts expressed in percent of the injected dose were: stomach, 0.9 ± 0.1%; its contents, 0.01%; small intestine, 5.7 ± 0.6%; luminal contents, 0.2 ± 0%; cecum, 1.1 ± 0.1%; and colon, 0.8 ± 0.1%. These were all appreciably less than the values for the corresponding parts of rats kept on the kidney bean diet *(Figure 1c)*. Furthermore, the counts accumulated in or the dry weight/100 g dry body weight of the small intestine of clenbuterol-fed rats were not different from those found in the lactalbumin control.

In these experiments the total recovery of radioactivity in rat tissues was high and accounted for 89–95% of the injected label.

Discussion

The polyamines putrescine, spermidine, and spermine are natural growth factors. Through their involvement in signal transduction and in nearly every step of DNA, RNA, and protein synthesis, polyamines are essential for cell proliferation. Because cell turnover in the intestinal epithelium is higher than in most other parts of the body, polyamines are vital for the proper structure and function of the entire digestive tract. Spermidine and spermine have also been implicated in the maturation of intestinal tissue.[18]

By causing damage and/or inducing growth, antinutritional factors (lectins, tannins, saponins) present in food of plant origin can influence the metabolic activity and increase the polyamine requirement of the gut. It is essential that this increased demand for polyamines be met so that the integrity and proper functioning of the gut are maintained.

According to earlier beliefs, most of the polyamines needed for adaptational growth are synthesized in situ by the gut.[19,20] However, in lectin-induced intestinal growth, most of the polyamines that accumulated in the small bowel were of extracellular origin,[5,7] coming, at least in part, from the food consumed. Indeed, all types of food, whether of plant (vegetables and fruit) or animal origin (milk, eggs, meat), were found to contain polyamines in varying amounts *(Table 1)*. This is not surprising, because polyamines are omnipresent components of all living cells.

There were interesting differences in the polyamine composition of the two major types of food, from plants and animals. Products prepared from meat or red meat contained spermine as the major polyamine component, while fish had more putrescine than spermidine or spermine. The polyamine composition of chicken meat was similar to that of pork and beef. The dominant polyamine in milk was spermidine. Cheese, a product of microbial fermentation, had high putrescine and spermidine contents.

Fruits were rich in putrescine and spermidine, but of the samples analyzed, only pears contained any spermine. The dominant polyamine in vegetables was either putrescine or spermidine.

On the basis of these results, typical diets for humans could supply hundreds of μmols of polyamines per day. Under normal conditions this should cover most of our needs, particularly as dietary polyamines are readily absorbed by the small intestine and pass into the systemic circulation.[5,7] However, there is evidence that a variable proportion of food polyamines is converted by the action of gut enzymes to polyamine- and/or non-polyamine metabolites before and during passage through the intestine.[5,7] Thus, the true amounts of polyamines reaching the body might be different for individual polyamines.

Of the catabolic enzymes, diamine oxidase[21] and polyamine-oxidase[22] seem to be the most important in limiting the availability of polyamines for absorption. For example, 1 hr after rats were intubated with ^{14}C-labeled putrescine, only 29–39% of the label was found as polyamines, with 11–15% remaining as putrescine.[7] This intensive breakdown of putrescine was probably the result of the action of diamine oxidase, which is one of the most abundant enzymes in the intestinal tissue. Spermidine and spermine were more highly conserved; 79% of spermidine and 72–74% of spermine from the food were recovered in their original form, as it was found earlier.[7] If conversion to the other two natural polyamines is taken into account, 87–96% of the labeled spermidine and 79–82% of spermine was conserved in polyamine form.[7] Accordingly, as spermidine and spermine were well conserved for further utilization in the body, they are the "right polyamines" to be absorbed from food in contrast to putrescine, which is mostly converted to non-polyamine metabolites.

Every organ of the body requires polyamines for growth, renewal, and metabolism. To determine if it is possible to induce changes in the distribution patterns of polyamines in the body of the rats by selec-

tively stimulating the growth of particular organs with different dietary factors, the animals were intraperitoneally injected with ^{14}C-spermidine after pre-feeding them on different diets. Because measurement of the kinetics of absorption and catabolism of intragastrically administered polyamines is technically difficult, results on the distribution of luminal polyamines are scanty. However, when comparisons have been made,[7] the luminal and intraperitoneal routes of administration of spermidine yielded similar results. Accordingly, for the establishment of the effects of food on the distribution of polyamines in the body of rats fed different diets, the preferred route of administration was by intraperitoneal injection.

As expected, the metabolic activity of different organs was highly influenced by the addition of different growth stimulants to the diet. Similarly, the three dietary treatments resulted in the accumulation of significantly different amounts of polyamines in the organs targeted by these growth factors *(Figure 1 a,b,c)*. For example, during PHA-induced growth of the gut, the small intestine increased both its weight and polyamine content by about 60%.[23] Similarly, with the loss of skeletal muscle,[15,16] its polyamine content also decreased.[23] In accordance with these findings, the changes in the distribution patterns of intraperitoneally injected ^{14}C-spermidine in the same organs *(Figure 1)* were well correlated with changes in tissue weight and polyamine content. Because more radioactivity accumulated in the digestive tract and less in the atrophied skeletal muscle of rats fed the kidney bean diet (in comparison with the lactalbumin controls), measurement of the distribution of labeled spermidine in the body appears to be a sensitive indicator of food-induced shifts in metabolism. The increased accretion of polyamines in hypertrophied skeletal muscle of rats treated with clenbuterol and the lack of significant changes in polyamine concentrations in their unaffected gut[23] were also well correlated with the distribution patterns of the intraperitoneally injected labeled spermidine *(Figure 1)*.

These results are in accord with the suggestion that exogenous polyamines play an important role in the growth process of tissues and organs. Although every cell has some capacity to synthesize polyamines, it appears that the body also relies on a continuous supply of polyamines from the food, most of which are not retained by the gut tissues but distributed in different organs of the body. Thus, the polyamine requirements that cannot be met by biosynthesis have to be satisfied by exogenous polyamines derived from the food. It is likely that there is a body storage pool for polyamines from which they are released in a controlled way when needed. This process is probably regulated by hormones and growth factors.[24]

Muscle is the general energy and nutrient store of the body. It can be filled up relatively easily and also has the capacity to release stored nutrients in a controlled way.[23] The results presented here suggest that muscle (and skin) might be an important part of the putative body polyamine pool.

The basic information presented in this paper is regarded only as a start. It is not clear, for example, what effect handling and preparation of food has on the quality and quantity of polyamines and their bioavailability. More information is urgently needed on food polyamine content and composition, and daily inputs on various standard diets should be calculated. The size and composition of body polyamine pools in health or pathological states have to be established and related to polyamine biosynthesis, intake, and storage. Such studies are currently in progress in our laboratory.

Abbreviations

ODC	Ornithine decarboxylase
DFMO	α-difluoro-methylornithine
PHA	phytohemagglutinin, the lectin from kidney bean *(Phaseolus vulgaris)*
SAM DC	S-adenosyl-L-methionine decarboxylase

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